REMARKS

I. Status of the Claims

Claims 1-40 are pending in the application. Claims 30-40 are withdrawn pursuant to a restriction requirement. Thus, claims 1-29 are under examination and are rejected as follows: claims 1-3 under 35 U.S.C. §112, second paragraph; claims 1-6, 16, 24, 28 and 29 under 35 U.S.C. §102(b) over Johnsson and Varshavsky (hereafter "Johnsson"); claims 1, 4, 7-9, 16-18 and 26 under 35 U.S.C. §102(e) over Michnick *et al.* (hereafter "Michnick"); claims 1, 4, 9 and 10-12 under 35 U.S.C. §102(b) over Rossi *et al.* (hereafter "Rossi"); claim 13 over Johnsson in view of Zwick *et al.* (hereafter "Zwick"); claims 14 and 15 over Johnsson in view of Farzaneh *et al.* (hereafter "Farzaneh"); and claims 19-23, 25 and 27 over Johnsson. The specific grounds for rejection, and applicants' response thereto, are set out in detail below.

II. Telephonic Interview

Applicants and their representative appreciate the PTO's examining and supervising personnel taking the time to conduct an interview regarding the Office Action and the claims. Applicants' response, in addition to addressing issues raised in the action, also seeks to address clarity concerns raised during the interview. Should any issues have been overlooked, or should new issues arise, a telephone call to the undersigned is welcomed.

III. Restriction/Election of Species

Applicants hereby affirm their election of Group I, claims 1-29, and their elected species of β-galactosidase (claim 10) and maltose binding protein (claim 13). These elections are made

without traverse. All claims read on the elected species; all claims are generic with respect to maltose binding protein; all claims other than claim 11 are generic with respect to β-galactosidase.

IV. Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 1-3 are rejected under the second paragraph of §112. Amendments have been provided to clarify claim 1 with respect to each of the stated concerns. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

V. Rejections Under 35 U.S.C. §102

A. §102(b) over Johnsson

Claims 1-6, 16, 24, 28 and 29 stand rejected under §102(b) over Johnsson. Applicants respectfully traverse.

The concept behind the Johnsson reference is quite different than that of the present invention. In the present invention, there is a first marker protein segment linked to a protein of interest. There also is a second marker protein segment. When the protein of interest is properly folded/soluble, the first and second marker segments combine, as they would normally, and the resulting complex becomes "functional," allowing one to assess the degree of complex formation, which reflects the folding/solubility status of the fused protein of interest, by measuring the amount of marker protein function. If the protein of interest is ***not*** properly folded/soluble, the availability of the first marker protein segment is negatively impacted by its fusion partner (the protein of interest), and its combination with the second marker protein

segment is inhibited. Thus, examining the extent of marker complex formation, determined by measuring a function exhibited by the marker segments only when associated, is a proxy for the folding of the protein of interest.

On its face, Johnsson might appear similar to the present invention, but a close review of the reference reveals a distinct difference. According to the examiner, ubiquitin constitutes the marker protein – having N- and C-terminal portions that combine to form a "functional" complex. Assuming that is true, the ubiquitin subunits cannot also constitute "a protein of interest." Rather, the only "protein of interest" identified by the action is "the leucine zipper homodimerization domain of *S. cerevisiae* Gcn4." It is here that the examiner's reasoning begins to unravel.

The leucine zipper homodimerization domain of *S. cerevisiae* Gcn4 is not a "protein of interest" as set forth in the present invention. The Johnsson authors admittedly had no interest whatsoever in the folding or solubility of this peptide. Looking at the ***teachings of the reference as a whole***, we see that the ***reason*** for including the leucine zipper domain was to set up what the authors describe as the USPS (ubiqitin-based split protein sensor) assay. In order for this assay to work, ***each segment of ubiquitin is linked to a member of a protein pair that is capable of interaction***: "[I]f C_{ub} and the altered N_{ub} [mutated to prevent normal interaction] are each linked to polypeptides that interact *in vivo*, the cleavage of the fusion containing C_{ub} is restored ...." Johnsson, abstract. Thus, the only reason to have the leucine zipper homodimerization domain on the N_{ub} segment is if there is to be a similar domain on the C_{ub} segment. The interaction of these two proteins drives the reconstitution of the ubiquitin complex, thereby allowing ubiquitinases to cleave off the reporter fragment. Thus, the leucine zipper homodimerization

domain cannot be interpreted as "a protein of interest" for which information on solubility/folding is desired unless one ignores the teachings of Johnsson ***as a whole***.

Moreover, in the present invention, an interaction by moieties ***other*** than the marker segments would defeat the entire purpose of the invention, *i.e.*, to assess proper folding/solubility. In other words, if the protein of interest somehow facilitated the interaction of the two marker segments (one of which being its fusion partner), it would reduce the sensitivity of the entire assay, ***an assay designed to assess changes in folding/solubility by looking at changes in the ability of the marker segments to combine and form a functional complex.*** This goal stands in stark contrast to that of Johnsson, discussed above, where "artificial" association of marker segments is promoted, and natural association of the marker segments is "mutated out" of their system. These are fundamental differences that preclude a holding of anticipation.

While not necessary in light of the foregoing explanation, applicants are providing an amendment that more clearly sets forth and claims these distinctions. Specifically, applicants are amending step (b) of claim 1 to recite a polypeptide including the second marker segment "consisting essentially of" this segment, *i.e.*, it does not contain any other sequences that materially affect the characteristics of this molecule in the context of the claimed invention.[1] Thus, even if the leucine zipper homodimerization domain is considered a "protein of interest" on N_{ub}, the inclusion of the same domain on C_{ub} clearly would not satisfy the limitation of not materially affecting the characteristics of that molecule.

[1]"Consisting essentially of" has been interpreted by the courts as "partially open" language. Thus, the claim is open for inclusion of unspecified ingredients that do not materially affect the basic and novel characteristics of the claimed composition. *Carter-Wallace Inc. v. The Gillette Co.*, 211 USPQ 499, 527, n. 29 (D.Mass 1981).

In sum, though a number of features in Johnsson appear similar to those in the claimed invention, there are in fact distinct differences – differences that do not permit a rejection for anticipation to stand. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

B. §102(e) over Michnick

Claims 1, 4, 7-9, 16-18 and 26 stand rejected under 35 U.S.C. §102(e) over Michnick. Applicants submit that, for the same reasons advanced above with respect to Johnsson, this reference also fails to anticipate.

As discussed by the examiner, the Michnick patent discloses fusions between marker segments and the same leucine zipper homodimerization domain of *S. cerevisiae* Gcn4 described in Johnsson. Again, however, Michnick expressed no interest whatsoever in the folding or solubility of the the leucine zipper homodimerization domain. Rather, this is merely a vehicle to demonstrate the feasibility of using pairs of interacting proteins ***that are distinct from the marker segments***. Notably, Michnick states that "It is crucial to understand that these assays will only work if the fused, interacting proteins catalyze the reassembly of the enzyme." Column 4, lines 38-42. Clearly, as stated by Michnick, the enzyme itself cannot facilitate reassembly, ***yet that is the very premise upon which the present invention is based.*** Applicants again point to the amendment introducing "consisting essentially of" into step (b) of claim 1. This further highlights the distinction between the claimed invention, where the second polypeptide cannot contain other, non-marker sequences capable of interacting with the first polypeptide.

As with Johnsson, though a number of features in Michnick appear similar to those in the claimed invention, there are differences that do not permit a rejection for anticipation to stand. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

C. §102(b) over Rossi

Claims 1, 4, 9 and 10-12 stand rejected under 35 U.S.C. §102(b) over Rossi. Once again, though the examiner has identified common features between the claimed invention, and those of the reference, applicants submit that important differences exist.

Just as in Michnick and Johnsson, the system disclosed by Rossi is reliant not on the interaction of two marker segments, but on the interaction of the fusion partners for each of the marker segments, *i.e.*, the test protein. The examiner readily acknowledges that in quote from the reference: "Rossi discloses that for this method it is essential 'to select [beta-galactosidase subunits] with sufficiently low affinity for each other so that they monitored rather than drove the association of the test proteins.'" Office Action at page 12. ***This is the exact opposite of the present invention, where the ability of the two marker segments to interact on their own is a requirement.*** Thus, again, the introduction of "consisting essentially of" in the instant claims clarifies that Rossi's system is ***not*** the same, as Rossi's second polypeptide clearly contains other sequences (a second test protein) that materially affect the way the second polypeptide interacts with the first.

Rossi, though disclosing a number of features similar to those in the claimed invention, is further characterized by distinct differences, and these differences preclude a finding of

anticipation. Reconsideration and withdrawal of the rejection is, therefore, respectfully requested.

VI. Rejections Under 35 U.S.C. §103

A. Johnsson in view of Zwick

Claim 13 stands rejected under §103 over Johnsson in view of Zwick. Johnsson is cited as above. Zwick is cited as teaching fusion of a protein of interest to maltose binding protein, and that this "was known to improve the solubility of the protein." Applicants traverse.

As discussed above, Johnsson does not provide the teaching necessary to anticipate any claims of the present invention. For the same reason, it fails as a primary reference in rejecting claim 13, even in light of Zwick's teachings regarding maltose binding protein.

For the foregoing reasons, claim 13 is not obvious over Johnsson and Zwick. Reconsideration of the rejection is therefore respectfully requested.

B. Johnsson in view of Farzaneh

Claims 14 and 15 stand rejected under §103 over Johnsson in view of Farzaneh. Johnsson is cited above. The examiner cites Farzaneh as teaching integrated and episomal foreign nucleic acid. Applicants traverse.

As discussed above, Johnsson does not provide the teaching necessary to anticipate any claims of the present invention. For the same reason, it fails as a primary reference in rejecting claims 14 and 15, even in light of Farzaneh's teachings regarding maltose binding protein. Thus,

as discussed above, claims 14 and 15 are not obvious over Johnsson and Farzaneh. Reconsideration of the rejection is therefore respectfully requested.

C. Johnsson

Claims 19-23, 25 and 27 stand rejected under §103 over Johnsson. Johnsson is cited as above, but admittedly fails to teach specific promoters and specific host cells, each of which is said to be "well known in the art" at the time the application was filed. However, as explained above, Johnsson fails to provide the teachings necessary to anticipate or render obvious each of the rejected claims. Thus, reconsideration of this rejection is also respectfully requested.

VI. Conclusion

In light of the foregoing, applicants submit that all claims are in condition for allowance, and an early indication to that effect is earnestly solicited. Should the examiner have any questions regarding this response, a telephone call to the undersigned is invited.

Please date stamp and return the enclosed postcard as evidence of receipt.

Respectfully submitted,

Steven L. Highlander
Reg. No. 37,642
Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-3184

Date: January 16, 2003

APPENDIX A: MARKED UP COPY OF CLAIMS

1. (Twice amended) A method for assessing [protein] folding and/or solubility of a protein of interest comprising:

   a) providing an expression construct comprising (i) a gene encoding a first polypeptide [fusion protein, said fusion protein] comprising [a] said protein of interest fused to a first segment of a marker protein, wherein said first marker segment has only systematic effects on the folding or solubility of the protein of interest, and (ii) a promoter active in [said] a selected host cell and operably linked to said gene;

   b) expressing said [fusion protein] first polypeptide in [a] said host cell that also expresses a second [segment] polypeptide consisting essentially of a second segment of said marker protein, wherein said second marker segment [is capable of structural complementation] combines with said first marker segment to form a functional marker protein when said first polypeptide is properly folded; and

   c) [determining structural complementation] assessing the ability of said first and second marker segments to combine to form a functional marker protein,

   wherein a greater degree of [structural complementation] marker function, as compared to [structural complementation] marker function observed with appropriate negative controls, indicates [proper] improved folding and/or solubility of said protein of interest.

2. (Amended) The method of claim 1, wherein said [fusion is] first polypeptide comprises said first marker segment fused C-terminal to said protein of interest.

3. (Amended) The method of claim 1, wherein said [fusion is] first polypeptide comprises said first marker segment fused N-terminal to said protein of interest.

12. (Amended) The method of claim 11, wherein said first marker segment is the α-peptide of β-galactosidase, and said second segment is the ω-peptide of β-galactosidase.

14. (Amended) The method of claim 1, wherein a gene encoding said second [segment] polypeptide is carried on a chromosome of said host cell.

15. (Amended) The method of claim 1, wherein a gene encoding said second [segment] polypeptide is carried episomally in said host cell.

28. (Amended) The method of claim 1, wherein said negative control utilizes a host cell lacking the second [segment of said marker protein] polypeptide.

APPENDIX B: CLEAN COPY OF PENDING CLAIMS (UNOFFICIAL)

1. A method for assessing folding and/or solubility of a protein of interest comprising:

   a) providing an expression construct comprising (i) a gene encoding a first polypeptide comprising said protein of interest fused to a first segment of a marker protein, wherein said first marker segment has only systematic effects on the folding or solubility of the protein of interest, and (ii) a promoter active in a selected host cell and operably linked to said gene;

   b) expressing said first polypeptide in said host cell that also expresses a second polypeptide consisting essentially of a second segment of said marker protein, wherein said second marker segment combines with said first marker segment to form a functional marker protein when said first polypeptide is properly folded; and

   c) assessing the ability of said first and second marker segments to combine to form a functional marker protein,

   wherein a greater degree of marker function, as compared to marker function observed with appropriate negative controls, indicates improved folding and/or solubility of said protein of interest.

2. The method of claim 1, wherein said first polypeptide comprises said first marker segment fused C-terminal to said protein of interest.

3. The method of claim 1, wherein said first polypeptide comprises said first marker segment fused N-terminal to said protein of interest.

4. The method of claim 1, wherein said marker protein is selected from the group consisting of a target binding protein, an enzyme, a protein inhibitor, a fluorophore and a chromophore.

5. The method of claim 4, wherein said marker protein is a target binding protein.

6. The method of claim 5, wherein said target binding protein is ubiquitin.

7. The method of claim 4, wherein said marker protein is a chromophore.

8. The method of claim 7, wherein said chromophore is green fluorescent protein, blue fluorescent protein, yellow fluorescent protein, luciferase or aquorin.

9. The method of claim 4, wherein said marker protein is an enzyme.

10. The method of claim 9, wherein said enzyme is β-galactosidase, cytochrome c, chymotrypsin inhibitor, Rnase, phosphoglycerate kinase, invertase, staphylococcal nuclease, thioredoxin C, lactose permease, amino acyl tRNA synthase, and dihydrofolate reductase.

11. The method of claim 10, wherein said enzyme is β-galactosidase.

12. The method of claim 11, wherein said first marker segment is the α-peptide of β-galactosidase, and said second segment is the ω-peptide of β-galactosidase.

13. The method of claim 1, wherein said protein of interest is Alzheimer's amyloid peptide (Aβ), SOD1, presenillin 1 and 2, α-synuclein, amyloid A, amyloid P, CFTR, transthyretin, amylin, lysozyme, gelsolin, p53, rhodopsin, insulin, insulin receptor, fibrillin, α-ketoacid dehydrogenase, collagen, keratin, PRNP, immunoglobulin light chain, atrial natriuretic peptide, seminal vesicle exocrine protein, β2-microglobulin, PrP, precalcitonin, ataxin 1, ataxin 2, ataxin 3, ataxin 6, ataxin 7, huntingtin, androgen

receptor, CREB-binding protein, dentaorubral pallidoluysian atrophy-associated protein, maltose-binding protein, ABC transporter, glutathione S transferase, and thioredoxin.

14. The method of claim 1, wherein a gene encoding said second polypeptide is carried on a chromosome of said host cell.

15. The method of claim 1, wherein a gene encoding said second polypeptide is carried episomally in said host cell.

16. The method of claim 1, wherein said host cell is selected from the group consisting of a bacterial cell, an insect cell, a yeast cell, a nematode cell, and a mammalian cell.

17. The method of claim 16, wherein said host cell is a bacterial cell.

18. The method of claim 17, wherein said bacterial cell is *E. coli*.

19. The method of claim 18, wherein said promoter is the *Taq* promoter; T7 promoter, or the P_{lac} promoter.

20. The method of claim 16, wherein said host cell is a nematode cell.

21. The method of claim 20, wherein said nematode cell is a *C. elegans* cell.

22. The method of claim 16, wherein said host cell is an insect cell.

23. The method of claim 22, wherein said host cell is a *S. fugeria* cell.

24. The method of claim 16, wherein said host cell is a yeast cell.

25. The method of claim 14, wherein said promoter is CupADH or Gal.

26. The method of claim 16, wherein said host cell is a mammalian cell.

27. The method of claim 26, wherein said promoter is PepCk or tk.

28. The method of claim 1, wherein said negative control utilizes a host cell lacking the second polypeptide.

29. The method of claim 1, wherein said negative control utilizes a fusion protein that is improperly folded and/or insoluble.